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Our Case No. 8642/88-1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

NABEL *et al.*

Serial No. 09/458,610

Filing Date: December 10, 1999

For TREATMENT OF DISEASES BY SITE SPECIFIC INSTILLATION OF CELLS OR SITE SPECIFIC TRANSFORMATION OF CELLS AND KITS THEREFOR

Examiner: Wehbé

Group Art Unit No.: 1632

DECLARATION OF ELIZABETH G. NABEL UNDER 37 C.F.R. § 1.132

I, Elizabeth G. Nabel, declare that:

1. I received a B.A. in psychology from St. Olaf's College and graduated from Cornell University Medical College with an M.D. in 1981. I was a resident in internal medicine and a fellow in hypertension and cardiology at Brigham and Women's Hospital from 1984-1987. In 1987, I became an assistant professor of internal medicine at The University of Michigan Medical Center and rose to professor and Chief of the Division of Cardiology before leaving in 1999. Since 1999, I have been the Scientific Director of Clinical Research at the National Heart, Lung and Blood Institute in Bethesda, Maryland.

2. I am a co-inventor of the above-identified patent application ("the '610 application"). I have read and understood the Office Action dated and

October 30, 2002. I have been told and understand that th claims of the '610 application have been rejected for lack of enablement.

3. The claimed subject matter is directed to a method of introducing protein in a mammal. As set forth in the claims and as taught in the specification, that method is accomplished by delivering transformed vascular cells to a blood vessel of the mammal, wherein the transformed cells originate from the mammal, or are syngeneic to the mammal. The transformed cells further contain an exogenous nucleic acid that encodes the desired protein and are competent to express that protein when delivered to a mammalian blood vessel in accordance with the claimed invention. *See, e.g.*, Claim 106.

4. It is my understanding that the Examiner asserted that the '610 application, while establishing that instillation of cells transformed with a marker gene into a blood vessel lead to expression of the marker gene in that blood vessel, nevertheless, failed to demonstrate "expression of a therapeutic levels of protein or the treatment of any disease or condition" (Office Action, October 30, 2003, page 5, top paragraph).

5. Following the teaching and guidance of the '610 application, and under my direction, the work described below demonstrates the transformation of vascular smooth muscle cells (VSMCs) from a pig with an adenovirus vector encoding the protein p27. Those transformed cells were instilled into a balloon-injured artery of the same pig and expressed sufficient p27 to inhibit intimal hyperplasia. Hence, this work demonstrates that expression of

therapeutic levels of protein suffici nt to treat a cardiovascular condition can b achieved in accordance with the claimed subj ct matter by following the teaching and guidance of the '610 application.

6. In particular, this work was conducted as described at pages 14-16 of the '610 application.

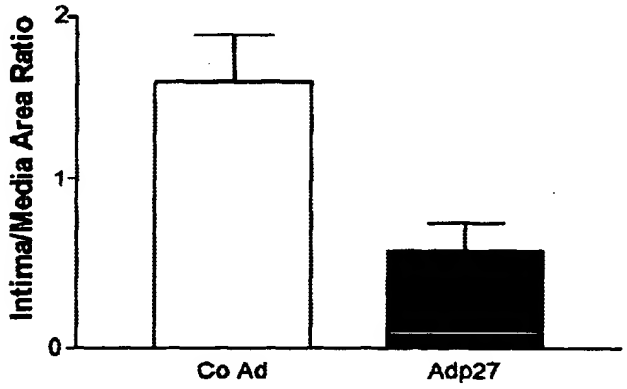
7. Four pigs, two as a control group and two as an experimental group, were used. In each pig, VSMCs were isolated from a peripheral vein and grown in cell culture. The cultured VSMCs from two pigs were transformed with an adenoviral vector expressing p27, a cell cycle inhibitor protein (Adp27). The cultured VSMCs from the two control pigs were transformed with a control adenoviral vector that does not express a biologically active protein (AdCo). Four days after transformation, the cells were examined by immunostaining for p27 and Western blotting and confirmed that the Adp27 cell lines expressed p27 and the cell lines in the control group did not.

8. Thereafter, the transformed VSMCs were site-specifically instilled into the pigs. Each of the four pigs was anesthetized and the two femoral arteries were exposed. A balloon angioplasty catheter was introduced into each femoral artery, and the balloon was inflated to create a vascular injury. Following vascular injury of each artery, each arterial segment was flushed with saline, and 4.5×10^6 transformed VSMCs were instilled therein at the site of injury. The transformed VSMCs originated from the pigs in which they were implanted.

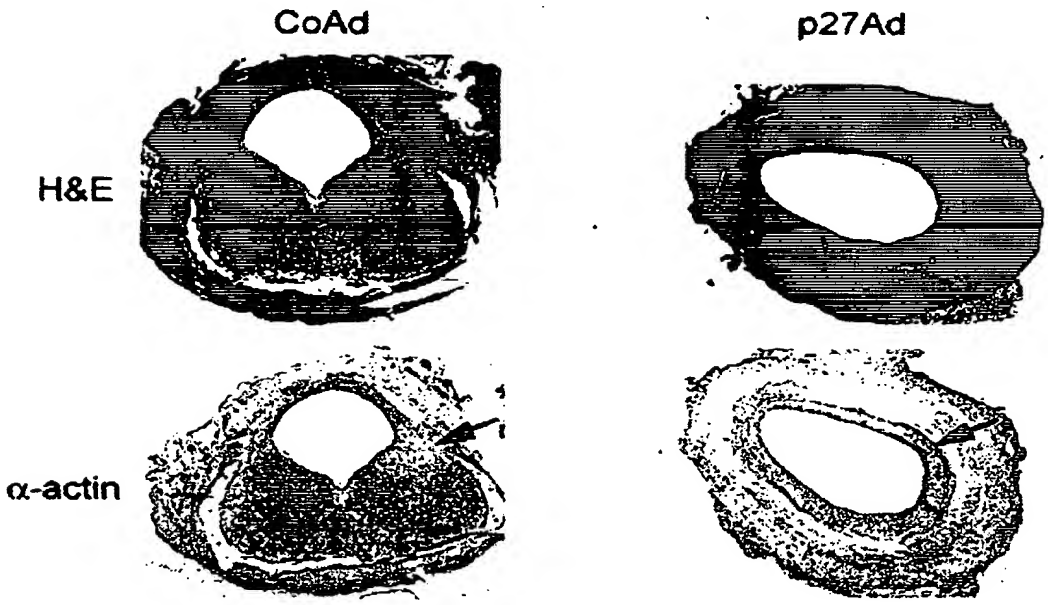
9. The pigs were allowed to recover for three weeks. Following the recovery period, the pigs were anesthetiz d, the arterial segments were removed from each pig, and the pigs were euthanized. The arterial segments were fixed and analyzed.

10. Analysis of the arterial segments revealed that the two experimental pigs, with VSMCs expressing p27, had a significant reduction in intimal hyperplasia and arterial lesion development as compared to the two control pigs.

11. Below is a graph depicting the intima/media area ratio in arterial segments of the control group (CoAd) and those of the experimental group (Adp27). The intima/media ratio in the experimental group (Adp27) was 0.58+0.17 and was significantly reduced (P<0.01) compared to the intima/media ratio in the control group (CoAd), which was 1.61+0.29.



12. Depicted below are photomicrographs of representative sections of the arterial segments stained either with h matoxylin and eosin (H & E) or α-actin stains. Th arrows indicat the elastic lamina. In the photomicrographs, severe media necrosis is observed in the control treated (CoAd) pigs, whereas media necrosis is absent in the experimental pigs (p27Ad).



13. These results demonstrate that transformed cells, originating from the mammal to which the cells were delivered and competent to express p27 (a therapeutic protein), were instilled into an arterial segment and expressed p27 at levels sufficient to inhibit to intimal hyperplasia in injured arteries in accordance with the teaching and guidance of the '610 application and the claimed subject matter.

14. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on knowledge and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the U.S. Code and that such willful false statements may jeopardize the validity of the patent application or any patent issuing thereon.

6/27/03
Date

Elizabeth S. Nabel
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